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Beat Frequency is Reduced but Waveform Shape is Conserved in Chlamydomonas Flagella at High Viscosity
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Motivation: Cilia and flagella are thin subcellular organelles that produce fluid movement and cell motility. Coordinated activity of the motor protein dynein causes sliding between adjacent microtubule doublets and subsequent bending of the cilium. Here, the role of mechanical feedback in dynein regulation is investigated by studying the effects of increased viscosity on the flagellar waveform.
Methods: Flagellar waveforms of the unicellular green alga Chlamydomonas reinhardtii were measured in media of different viscosities. Genetic mutants missing inner dynein arms (ida1) or outer dynein arms (oda2) were used to investigate the force-dependent contributions of these molecular motors to the flagellar waveform. Media viscosity was increased from 1.5 cP to 4.7 cP using Ficoll 10% w/v. High speed videos (350 fps) of periodically-beating uniflagellate cells were processed in MATLAB to extract waveform parameters sensitive to external viscosity, and to compare mutant cells to wild-type cells.
Results: Beat frequency decreased with increasing viscosity in wild-type (59.5 ± 7.0 Hz to 31.7 ± 9.1 Hz) and mutant cells (ida1: 50.4 ± 6.1 Hz to 27.3 ± 11.1 Hz; oda2: 24.9 ± 4.0 Hz to 7.2 ± 1.8 Hz). Both wild-type and oda2 cells exhibited greater maximum curvature at the higher viscosity (wild-type: 0.212 ± 0.042 rad/µm to 0.287 ± 0.025 rad/µm; oda2: 0.163 ± 0.033 rad/µm to 0.230 ± 0.014 rad/µm). Curvature differences were smaller in the ida1 mutant (0.266 ± 0.024 rad/µm to 0.288 ± 0.089 rad/µm). Shear rate and bend propagation speed decreased with viscosity, and the amplitude of shear was reduced slightly. Notably the qualitative shape of the waveform and relative differences between wild-type, ida1, and oda2 were maintained at the higher viscosity.
Discussion: At higher viscosity, beat frequency decreases to maintain viscous force amplitudes and flagellar waveforms similar to baseline, which supports the role of mechanical feedback in dynein coordination.

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Second Chance Mechanism Explains Dwell Time Distributions of Myosin and Dynein
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We reproduced experimental dwell time distributions of head and tail labeled dynein and myosin V at low and high [ATP] with a continuous time Markov chain (CTMC) that we developed using second chance mechanics (SCM) (doi: 10.1371/ journal.pone.0041098). SCM is a non-equilibrium kinetic model. If work is done by cycles of force acting on protein interactions, SCM determines the probability that the displaced interaction is restored before the proteins separate. For a two headed molecule with a chemo-mechanical cycle, one phase of the cycle displaces a head from its site of interaction while another phase provides a second chance for it to bind. A minimum CTMC consists of three observed states of the motor, i.e. unbound (C), bound by two heads (m_1), and bound with one head (m_2). The transition between m_1 and m_2 has unitary probability and marks a physical displacement. Other state transitions have stochastic probabilities determined by the time derivative of the CTMC at steady-state given a set of transition rates. Derived probabilities form the initial distribution of a Markov matrix based on the CTMC. We simulated the stationary probability distribution of the Markov matrix by a Monte Carlo scheme of CTMC. Each step of the CTMC includes a waiting time based on catalytic rates of bound or unbound motor domains and the waiting times of each step are exponentially distributed. The dwell times of the simulated CTMC are gamma distributed because a minimum of two steps is required to complete a dwell period. Hence, the gamma distributed dwell times of myosin V and dynein may be explained by a series of delays, i.e.one to wait for favorable binding of the unbound head and another to wait for the displacement of a bound head.

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The Role of the Cortex and the Cytoplasm in Deformations of the Plasma Membrane
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Recently, the role of the actin cortex has been shown to play a considerable role in resisting mechanical deformations of the membrane. Not only does this membrane-tethered network withstand large magnitude, high-aspect ratio, localized loads, it also responds to deformations by aiding in recovering their pre-deformed morphologies. Cytoplasmic flow, has also recently gained attention, and may aid in this shape-recovery process. Considered a biphasic material, the cytoplasm has been shown to manoeuvre its liquid cytosol through its solid filamentous networks, in response to force. Here, we examine the short and long-term dynamics of membrane-cortex deformations, and investigate the influence of the membrane, cytoskeletal components, and osmotic pressure. Atomic force microscopy was used to induce localized cellular deformations while resonant scanning laser confocal microscopy was used to quantify and observe the deformation and recovery response within the plane of deformation. Following both short (seconds) and long (minutes) perturbations, HeLa cells displayed two distinct responses: cells either recovered immediately (milliseconds), or gradually (seconds). While 90% of untreated cells recovered quickly, anti-cytoskeletal inhibitors led to a drastic reduction in this response (only 10-20% fast recovery), demonstrating the main role of actin and microtubule networks in the recovery process. Despite cytoskeletal disruption, this small percentage of cells still recovered quickly, suggestive of osmotic pressure-driven shape changes. By subjecting cells to hyper- and hypo-osmotic conditions, we demonstrated that cytosolic flow, in concert with an intact cytoskeleton, is largely responsible for the quick return to pre-deformed morphologies. Altogether, we demonstrate that the cytoskeleton and cytoplasm act in concert in the recovery of cell shape, and that there exists a duality in the recovery response of cells, regardless of the cell having an intact cytoskeleton, the reason for which remains unknown.

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Membrane Nanowaves in Single and Collective Cell Migration
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Cell migration within a tissue is a fundamental biological process. The mechanism of cell migration involves membrane ruffling at the leading cell edge that is rapidly induced in response to certain extracellular signals. Membrane ruffling is characterized by dynamically fluctuating movements of membrane protrusions like blebs, lamellipodia and filopodia driven by dynamic rearrangements of cytoskeleton components. Very interestingly, membrane waves were described in the recent years and introduced as a new mechanistic component in the understanding of cell motility. Cells have the ability to produce centripetally propagating waves on their membranes, which are traveling membrane undulations that persist over microns. We report the characterization of three-dimensional membrane waves for migrating single and collective cells and describe their propagation using wide-field optical profiling technique with nanometer resolution. We reveal the existence of small and large membrane waves the amplitudes of which are in the range of ~3-7 nm to ~16-25 nm respectively. For migrating single-cells, the amplitude of these waves is about 30 nm near the cell edge. Two or more different directions of propagation of the membrane nanowaves inside the same cell can be observed. After BMP-2 growth factor treatment, the migration velocity is increased. In this case, only one direction of propagation for the waves exists and the amplitudes of these waves are more than 80 nm near the cell edge. Furthermore for collective-cell migration, these membrane nanowaves are attenuated on the leader cells and poor transmission of these nanowaves to follower cells was observed. After BMP-2 treatment, the membrane nanowaves are transmitted from the leader cell to several rows of follower cells. For collectively migrating cells, the membrane nanowaves are shared by neighboring migrating cells. This mechanism gives a new view on how single and collective-cells can modulate their motility.

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Stress-Fibers Dictate Cellular Curvature & Force Exertion
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It has recently been found that environmental mechanical cues can regulate cell fate and behavior. However, a complete quantitative description of cellular contractility is still lacking. One prospective model to describe the cellular behavior in its environment is that of an active gel as developed by Bischofs et al. In that model the cell is described as a contractile entity in which the membrane and actin-cortex is mimicked by a line-tension that counteracts the homogeneously contractile interior. From this model, predictions on the shape of the cell membrane that depend on the exerted force were made. We performed high-resolution optical experiments on cells plated on micro-pillar arrays to this model. However, we found that force exertion is homogeneous, but predominant in the direction of stress-fibers. Our novel model agrees well with our experiments and shows that forces mediated by stress-fibers are 14 times higher than forces generated by homogeneous contractility.